EFFECTS OF CHRONIC AMPHETAMINE ON CENTRAL SEROTONERGIC AND DOPAMINERGIC SYSTEMS IN RATS OF VARIOUS AGES

by

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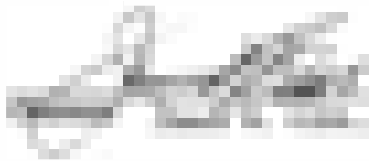
March 4, 1983  Ph.D.

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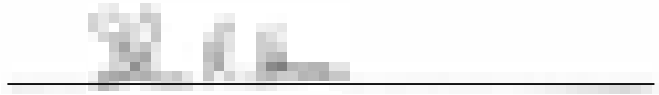
March 4, 1983 
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FINAL READING APPROVAL

To the Graduate Council of The University of Utah:

I have read the dissertation of Elaine Wyne Snowhill in its final form and have found that (1) its format, citations, and bibliographic style are consistent and acceptable; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the Supervisory Committee and is ready for submission to the Graduate School.

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ABSTRACT

The central neurochemical effects of chronic low doses (0.5 or 5 mg/kg, s.c., every 12 h for 2 wk) of d-amphetamine (AMPH) were studied in rats of varying ages. Tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) activities in neostriata of male offspring of dams exposed to 5 mg/kg AMPH during gestaton showed significant depressions at 3 weeks of age, while corresponding neurotransmitters were at control levels. In contrast, the striatal enzyme activities in the dams were normal, but significant depressions were observed in the levels of striatal 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA). All parameters returned to normal by 7 weeks. Neurochemical changes were minimal in juvenile and adult males subjected to the same AMPH dosing regimen. The hypothalamic concentrations of 5-HT, 5-HIAA, and DA in adult males were reduced at 16 h, but these parameters were unaffected in the juveniles. Recovery in the adults was complete by 3 wks post dosing. No effects were seen with the lower dose of AMPH (0.5 mg/kg) in any age group. The chronic doses of AMPH used in this study are not as toxic to the central serotonergic or dopaminergic systems of the rat as higher doses that have previously been studied in acute and subacute dosing regimens. Under these conditions, the age of the animal at the time of drug exposure did not significantly alter the severity of the toxicity that was ob-

served. Further, our data suggest that the neurotoxicity of AMPH is highly dependent on dose and duration of exposure.

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ACKNOWLEDGEMENTS

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INTRODUCTION

The neurochemical changes induced by high-dose, short-term exposure to amphetamines have been examined in great detail[1,9-12,14,15,17,18,33,38]. Multiple injections of methamphetamine (METH) (10-15 mg/kg/dose) over 18-36 hours cause severe depressions of the serotonergic and dopaminergic systems in several discrete brain regions of the rat[10,14,15,33]. This is not a generalized neurotoxic effect, as cholinergic and GABA-ergic systems in the same areas are not similarly affected[15]. Furthermore, striatal tyrosine hyroxylase (TH)[1,9] and tryptophan hydroxylase (TPH)[1] activities and 5-hydroxy-tryptamine (5-HT) levels[1] remain depressed as long as 110 days after dosing. Similarly, a single dose of amphetamine (AMPH) to iprindole-treated rats causes a decrease in striatal dopamine (DA) that persists at least 7 days[11,12,38].

Amphetamine-induced neurotoxicity has also been approached from a developmental standpoint. Wagner et al.[40] found that 10-day old rats exposed to very high doses of AMPH (25 mg/kg) twice daily for 30 days had depressed caudate DA levels at 2 weeks post-dosing. However, the magnitude of the change was significantly less than that previously reported in adult animals[39]. Lal and Feldmuller[20] calculated the whole brain half-life of AMPH in 12-day old rats to be 150 minutes, compared to 66 minutes in adults. In spite of this slower clearance, peak AMPH levels after a single injection of the drug were

found to be lower in the immature than in the adult animals[20].

High-dose acute or subacute studies are important in delineating the mechanisms of drug-induced toxicity and for providing animal models for the study of drug abuse. We were interested, however, in the neurochemical consequences of more clinically relevant AMPH exposure. AMPH is used therapeutically in low doses for the treatment of such diverse disorders as obesity, narcolepsy, and attention deficit disorder (ADD; i.e. minimal brain dysfunction, hyperkinesis)[41]. The latter condition, ADD, is most frequently recognized and treated in young children. The current research was designed to examine the neurochemical effects of chronic, low doses of AMPH and to determine whether any of the drug-induced changes are dependent on the age of the animal at the time of exposure.

MATERIALS AND METHODS

Adult male (180-200g), juvenile male (1-week old), and timed-pregnant female (day 2 of gestation) Sprague-Dawley rats were obtained from Simonsen Labs. Animals were housed in groups except for the pregnant females, which were housed separately. All rats were maintained in temperature-controlled rooms (24°C) and were allowed access to food and water ad libitum.

Experimental animals received d-amphetamine (0.5 or 5 mg/kg, s.c.) every 12 hours for two weeks; controls received equivalent volumes of the saline vehicle. Twice daily dosing with 0.5 mg/kg AMPH was selected for study as an approximation of the regimen used clinically in children for the treatment of ADD. The higher dose, 5 mg/kg, was included for purposes of direct comparison with the effects of the lower dose. Without assuming direct correlations between human use and our animal model, 5 mg/kg AMPH twice daily represents a dose level somewhat higher than is routinely used clinically in adults (e.g. narcolepsy, obesity). Higher doses could not be employed in this study due to an exceptionally high mortality rate in the pregnant females. Under the conditions described here mortality was less than 5 percent.

Figure 1 is a schematic representation of the dosing regimen and sacrifice schedule used in this study. Juvenile males were dosed beginning on postnatal day 14. Females were dosed from day 7 of gestation through day 20; the last dose was given 12-24 hours before par-

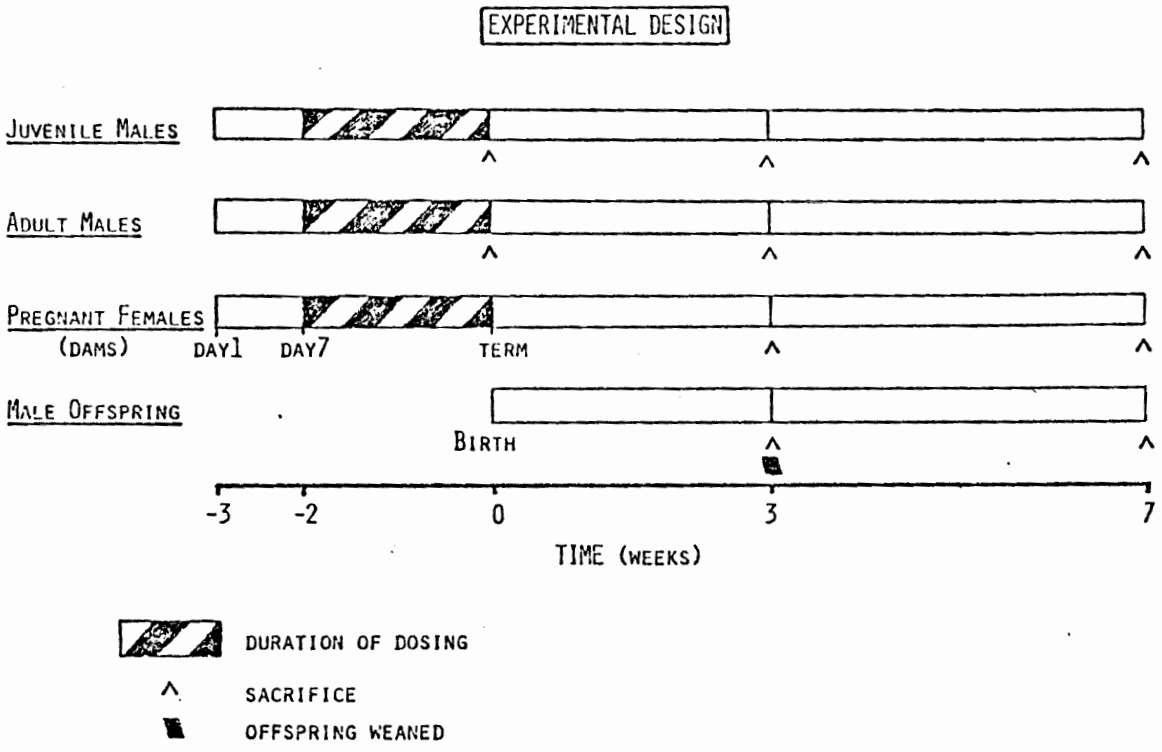

Figure 1. Experimental Design.

Amphetamine was administered subcutaneously every 12 hours for 2 weeks (shown by ▨). Offspring of the treated dams were weaned on postnatal day 21 (▮). Individual animals from each group were sacrificed at time points designated by ^. Sacrifice at Time 0 in the juvenile and adult males represents a time of 16 hours post-dosing.

turition. Male offspring of these dams, which were included in the neurochemical analyses, were exposed to AMPH in utero only. Individual animals from each group were sacrificed at the times shown in Figure 1. To allow ample differentiation of the brain for dissection, the earliest time of sacrifice of the offspring was three weeks. Females were required for nursing of the young until weaning at 21 days.

Rats were sacrificed between 09.00 and 12.00 hours to minimize the effects of diurnal and/or circadian rhythms on neurochemical activity. Rats were decapitated and the whole brain placed immediately on ice. Neostriatum, cerebral cortex, and hypothalamus were dissected out and frozen separately (-70 ^{o} C) in parafilm until analyses were performed. These brain regions were selected for study since the cortex and striatum have been reported to be particularly sensitive[1,14,23,33] and the hypothalamus more resistant[1,33] to the toxicity of the amphetamines. In addition, the developmental state of the hypothalamic hormonal influence was of interest for the comparison of the responses observed in the immature versus adult animals.

In neostriatum and cortex, enzyme activities were measured as follows: TPH activity by a modified $^{14}CO_2$-trapping method[16,37] as described previously by Hotchkiss et al.[15], TH activity by the method of Nagatsu et al.[25] In hypothalamus, as well as neostriatum and cortex, selected monoamines and metabolites were also measured. High performance liquid chromatography with electrochemical detection (HPLC-EC) was utilized to quantitate simultaneously 5-HT, 5-HIAA, DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA),

using a modification of the method described by Neilsen and Moore[27]. The HPLC conditions were as follows: Waters microbondapak C-18 reverse-phase analytical column (10 micron) with a mobile phase consisting of 0.1 M citric acid (anhydrous), 0.1 M potassium phosphate dibasic, 12.5% methanol, 0.004% sodium heptane sulfonic acid, and 1 mM EDTA, adjusted to a final pH of 3.5. Use of the present counter-ion in this procedure resulted in a different separation (Figure 2) than was previously described[27] using sodium octane sulfonic acid. Electrochemical detection (Bioanalytical Systems) was at a potential of +0.73V.

Brain regions were homogenized in the mobile phase buffer and centrifuged at 41,300 x g for 20 minutes. The supernatant was filtered across densely packed glass wool and a 50 µl aliquot injected directly onto the chromatography column. Quantitation of samples was achieved by comparison of peak heights to those of standard solutions injected daily.

The Student's t-test[6] was used to compare the appropriate control and treated groups, and in all cases a p value of < 0.05 was used to assign statistical significance.

Each of the paradigms were performed in a minimum of two separate experiments; the data were pooled, providing values of 12-18 animals per group unless otherwise stated.

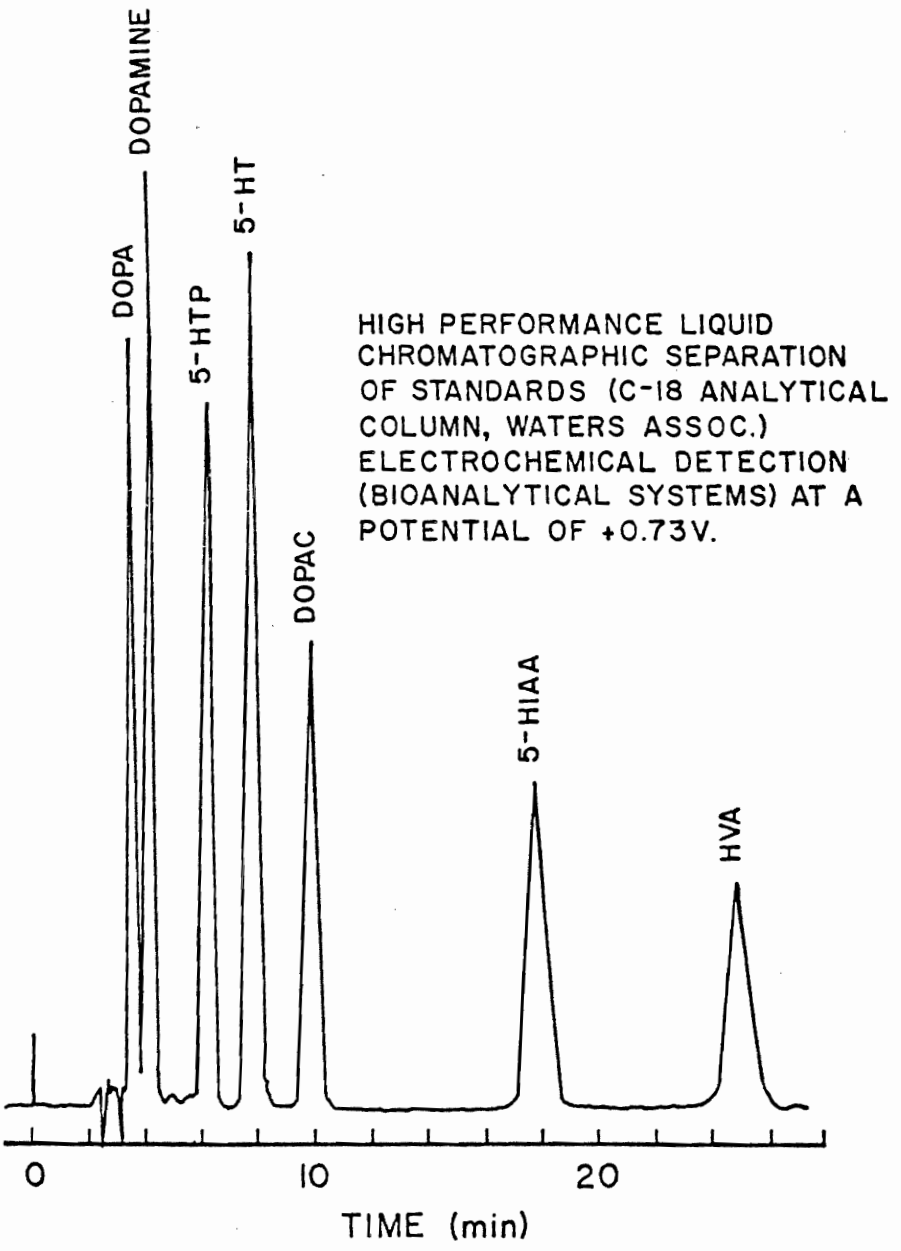

Figure 2. HPLC-EC separation of standards.

RESULTS

Most of the parameters measured exhibited a wide range of control values between age groups. Tables I and II give the mean absolute values (± S.E.M.) observed in adult male control animals. These figures are similar to those previously reported in the literature[1,29]. Except for a slightly enhanced striatal DA concentration ($p < .05$), values obtained in the control dams were not significantly different from those in the adult males (see Tables III and IV). Due to considerable postnatal development of monoaminergic systems in the rat[7,8,24,28], control values in the offspring and juvenile groups changed as a function of their age at the time of sacrifice (see Tables III and IV). Most of the dopaminergic parameters reached adult levels by 3 weeks of age, i.e., striatal TH, DOPAC, and HVA and hypothalamic DA. Striatal DA, however, did increase slightly between 3 and 7 weeks postnatally, indicating a lag period between maturation of the rate-limiting synthetic enzyme and its product. Most of the serotonergic parameters increased towards adult values during this time period (i.e., striatal and cortical TPH; striatal, cortical, and hypothalamic 5-HT). In contrast, 5-HIAA did not change with time in any of the areas studied. All parameters reached adult levels by 7 weeks of age, which is consistent with data reported elsewhere[24,28,31]. Because of these normal fluctuations in the absolute values of the parameters in control tissues, experimental data

Table I. Levels of 5-HT, DA, and metabolites (µg/mg tissue) in adult male control rats.†

	5-HT	5-HIAA	DA	DOPAC	HVA
Neostriatum	0.537 ± .010	0.522 ± .030	8.07 ± 0.25	1.10 ± 0.07	0.781 ± .063
Cortex	0.323 ± .019	0.178 ± .005	††	††	††
Hypothalamus	0.712 ± .015	0.698 ± .033	0.593 ± .034	††	††

†Values are expressed as means ± S.E.M.; (N = 12)
†† Not detected

Table II. TPH and TH activities (nMoles substrate oxidized/g tissue/hour) in adult male control rats.†

	TPH	TH
Neostriatum	32.5 ± 1.3	3173 ± 270
Cortex	29.3 ± 1.4	††

†Values are expressed as means ± S.E.M.; N = 12
††Not determined

Table III. Levels of 5-HT, DA, and metabolites (μg/mg tissue) of control rats.†

	5-HT	5-HIAA	DA	DOPAC	HVA
Dams					
Neostriatum	0.620 ± .030	0.545 ± .037	10.2 ± 0.4	1.10 ± .07	0.856 ± .040
Cortex	0.242 ± .011	0.201 ± .012	-	-	-
Hypothalamus	0.822 ± .041	0.632 ± .073	0.488 ± .051	-	-
Juvenile males					
16 h					
Neostriatum	0.476 ± .013	0.723 ± .064	5.72 ± .20	1.00 ± .046	0.767 ± .030
Cortex	0.183 ± .010	0.200 ± .011	-	-	-
Hypothalamus	0.720 ± .030	0.750 ± .022	0.601 ± .021	-	-
3 wk					
Neostriatum	0.606 ± .039	0.663 ± .060	8.70 ± .60	1.14 ± .09	0.747 ± .087
Cortex	0.222 ± .005	0.171 ± .011	-	-	-
Hypothalamus	0.830 ± .037	0.678 ± .028	0.625 ± .040	-	-
Male offspring					
3 wk					
Neostriatum	0.421 ± .024	0.781 ± .033	6.10 ± .15	0.950 ± .048	0.950 ± .060
Cortex	0.112 ± .012	0.177 ± .017	-	-	-
Hypothalamus	0.676 ± .072	0.696 ± .036	0.629 ± .036	-	-
7 wk					
Neostriatum	0.550 ± .020	0.760 ± .052	8.98 ± .31	0.976 ± .080	0.850 ± .081
Cortex	0.201 ± .010	0.175 ± .014	-	-	-
Hypothalamus	0.840 ± .024	0.640 ± .020	0.621 ± .031	-	-

† Values are expressed as means ± S.E.M.; N = 12 - 18
- Not detected

Table IV. TPH and TH activities (nMole oxidized/g tissue/hour) of control rats.†

	TPH	TH
Dams		
Neostriatum	28.3 ± 1.2	3140 ± 249
Cortex	28.2 ± 1.5	††
Juvenile males		
16 h		
Neostriatum	22.8 ± 1.9	2933 ± 167
Cortex	19.5 ± 1.4	††
3 wk		
Neostriatum	34.6 ± 1.8	2820 ± 187
Cortex	26.7 ± 1.4	††
Male offspring		
3 wk		
Neostriatum	21.2 ± 1.4	3041 ± 107
Cortex	19.2 ± 1.0	††
7 wk		
Neostriatum	30.6 ± 1.8	3111 ± 167
Cortex	24.3 ± 0.3	††

†Values are expressed as means ± S.E.M.; N = 12 - 18
††Not determined

throughout this discussion are expressed as percents of their corresponding controls.

The lowest dose of AMPH (0.5 mg/kg) caused no significant neurochemical changes at any time point tested (data not shown). The effects of 5.0 mg/kg AMPH in the neostriatum are shown in Tables V and VI. The dams were the only group to show uniform depressions in all monoamine and metabolite levels. At 3 weeks, the decreases were to approximately 80% of the control concentrations of 5-HT, 5-HIAA, DA, DOPAC, and HVA. The male offspring sacrificed at the same time showed only a slight depletion of DA (to 86%). Whereas striatal TPH and TH activities were normal in the dams, the enzymes were significantly depressed (to 73 and 80%, respectively) in their offspring. Although adult and juvenile males exhibited no changes 16 hours post dosing, TPH activity in the adults was increased to 134% of control at 3 weeks and recovered by 7 weeks.

There were few changes observed in the cerebral cortex of any of the groups (see Table VII). Adult males showed a significant depression of 5-HIAA (to 75% of control) at 16 hours which had recovered by 3 weeks. In addition, the only changes seen in this study at 7 weeks post-dosing (data not shown) were in the cortical 5-HT (80 ± 4%) and 5-HIAA (86 ± 3%) levels in juvenile-treated males (data not shown).

The hypothalamus was also relatively insensitive to the drug, with juvenile males showing more resistance than the adult-treated groups. At 16 hours post dosing, adult 5-HT, 5-HIAA, and DA were depressed to 79%, 68%, and 82% of controls, respectivly, while juvenile-

Table V. 5-HT, DA, and metabolite levels in neostriata of treatment groups at 16 h and 3 wk post-dosing (5.0 mg/kg AMPH, twice daily for two weeks).†

	5-HT	5-HIAA	DA	DOPAC	HVA
16 h					
Juvenile males	91 ± 7	98 ± 5	96 ± 4	95 ± 4	90 ± 4
Adult males	92 ± 4	97 ± 7	92 ± 4	101 ± 5	96 ± 6
3 wk					
Juvenile males	101 ± 6	98 ± 6	92 ± 4	92 ± 5	95 ± 5
Adult males	95 ± 3	96 ± 4	103 ± 5	105 ± 4	95 ± 7
Dams	83 ± 3*	83 ± 4*	81 ± 2*	80 ± 5*	79 ± 5*
Male offspring	96 ± 8	86 ± 11	86 ± 3*	95 ± 5	94 ± 6

† Values are expressed as mean percent of corresponding controls ± SEM; N =12-18 per group.

*Significantly different from control, $p < 0.05$

Table VI. TPH and TH activities in neostriata of treatment groups at 16 h and 3 wk post-dosing (5.0 mg/kg AMPH, twice daily for two weeks).†

	TPH	TH
16 h		
Juvenile males	96 ± 6	116 ± 6
Adult males	106 ± 7	109 ± 7
3 wk		
Juvenile males	101 ± 6	106 ± 5
Adult males	134 ± 8*	106 ± 5
Dams	106 ± 4	95 ± 6
Male offspring	73 ± 6*	80 ± 3*

† Values are expressed as mean percent of corresponding controls ± SEM; N = 12-18 per group.

* Significantly different from control, $p < 0.05$

Table VII. 5-HT and 5-HIAA levels and TPH activity in cerebral cortex of treatment groups at 16 h and 3 wk post-dosing (5 mg/kg AMPH, twice daily for two weeks).†

	5-HT	5-HIAA	TPH
16 h			
Juvenile males	90 ± 5	92 ± 6	98 ± 3
Adult males	90 ± 5	75 ± 7*	77 ± 10
3 wk			
Juvenile males	92 ± 6	89 ± 7	99 ± 4
Adult males	111 ± 6	110 ± 6	105 ± 5
Dams	88 ± 7	94 ± 8	84 ± 5
Male offspring	98 ± 8	104 ± 8	98 ± 6

†Values are expressed as mean percent of corresponding controls ± SEM; N = 12-18 per group

*Significantly different from control, $p < 0.05$

treated rats did not differ from their controls (see Table VIII).

To determine whether a larger dose of AMPH in this dosing regimen would induce more severe or longer-lasting neurochemical changes, one additional dosing experiment was performed (N = 6-8 per group). Juvenile and adult males were dosed with 10 mg/kg AMPH twice daily for 2 weeks (as described above). Pregnant females could not be used due to an exceptionally high mortality rate at this dose level. From these preliminary data (see Tables IX and X), a significant dose-effect component of AMPH-induced toxicity in this dosing paradigm was apparent. Quantitatively, more significant changes were seen in both groups at this higher dose. In the striata of juvenile males 5-HT and 5-HIAA were greatly depleted, being 64% and 68% of respective controls at 16 hours. These had completely recovered by 3 weeks, whereas smaller decreases in DA and HVA (each to 84% of controls) at 16 hours had not recovered by 3 weeks. Striatal TPH was elevated in the adult males at both time points and TH was significantly increased at 3 weeks, without accompanying changes in monoamine levels. Juvenile males exhibited striatal TH activity of 137% of control at 16 hours, with recovery to control by 3 weeks.

Cortical 5-HIAA was decreased in both groups at 16 hours; this was the only change observed in this brain region at the high (10 mg/kg) dose. Hypothalamic levels of 5-HT and DA that were depleted at 16 hours in adults and juveniles, respectively, had returned to control by 3 weeks.

Table VIII. 5-HT, 5-HIAA, and DA levels in hypothalamus of treatment groups at 16 h and 3 wk post-dosing (5 mg/kg AMPH, twice daily for two weeks).

	5-HT	5-HIAA	DA
16 h			
Juvenile males	94 ± 2	96 ± 3	90 ± 3
Adult males	79 ± 2*	68 ± 3*	82 ± 5*
3 wk			
Juvenile males	96 ± 3	95 ± 3	96 ± 3
Adult males	119 ± 8	128 ± 11	92 ± 10
Dams	88 ± 4	96 ± 3	96 ± 5
Male offspring	106 ± 7	101 ± 4	95 ± 5

† Values are expressed as mean percent of corresponding controls ± SEM; N = 12-18 per group.

*Significantly different from control, $p < 0.05$

Table IX. 5-HT, 5-HIAA, and DA levels and TPH activities in cerebral cortex and hypothalamus of juvenile- and adult-treated males at 16 h and 3 wk post-dosing (10 mg/kg AMPH, twice daily for two weeks).†

	5-HT	5-HIAA	DA	TPH
Cortex				
16 h				
Juvenile males	92 ± 4	75 ± 3*	-	98 ± 4
Adult males	89 ± 9	81 ± 5*	-	74 ± 12
3 wk				
Juvenile males	98 ± 5	114 ± 4	-	97 ± 3
Adult males	105 ± 6	115 ± 11	-	112 ± 9
Hypothalamus				
16 h				
Juvenile males	90 ± 3	81 ± 3*	87 ± 4*	††
Adult males	79 ± 2*	71 ± 4*	99 ± 9	††
3 wk				
Juvenile males	98 ± 1	98 ± 2	100 ± 7	††
Adult males	96 ± 6	104 ± 4	105 ± 4	††

† Values are expressed as mean percent of corresponding controls ± SEM; N = 6-8 per group

* Significantly different from control, $p < 0.05$

\- Not detected

†† Not determined

Table X. 5-HT, DA, and metabolite levels and TPH and TH activities in neostriata of juvenile- and adult-treated males at 16 h and 3 wk post-dosing (10 mg/kg AMPH, twice daily for two weeks),†

	5-HT	5-HIAA	DA	DOPAC	HVA	TPH	TH
16 h							
Juvenile males	64 ± 10*	68 ± 8*	84 ± 3*	88 ± 7	84 ± 5*	104 ± 3	137 ± 8*
Adult males	87 ± 3	98 ± 8	89 ± 5	106 ± 6	125 ± 9*	129 ± 9*	110 ± 7
3 wk							
Juvenile males	94 ± 2	96 ± 4	84 ± 3*	90 ± 3	85 ± 4*	104 ± 6	100 ± 4
Adult males	128 ± 25	95 ± 16	102 ± 8	123 ± 13	126 ± 13	124 ± 9*	119 ± 6*

†Values are expressed as mean percent of corresponding controls ± SEM; N = 6 - 8 per group

*Significantly different from control, $p < 0.05$

DISCUSSION

The control data document the developmental changes that occur in the dopaminergic and serotonergic systems in three discrete brain regions of the rat (Tables I, II, III, and IV). As both systems were monitored simultaneously in the same animals, several generalizations can be made. First, the neuronal systems studied here are not mature at birth in the rat, which is widely accepted. Secondly, different areas of the brain do not mature at the same rate. For example, hypothalamic DA was at adult levels by 3 weeks postnatally, whereas striatal DA reached mature levels only after 7 weeks. Finally, the different neurotransmitter systems do not mature at the same time; in the regions examined the dopaminergic system, generally, matured earlier than the serotonergic system. This has obvious significance for the development of any physiological functions, such as behavior, that depend upon the relative balance between such neurotransmitter systems for their expression.

The most striking observation from the experimental data is that AMPH, in this dosing regimen, caused only minimal, reversible neurochemical changes. This is in sharp contrast to the severe, long-lasting depressions of both the serotonergic and dopaminergic systems that have been observed after high-dose subacute drug exposure[1,9,14,33,39]. Short-term exposure to low doses of METH does not cause toxicity in these neurotransmitter systems[10,14]; a single dose of up

to 1 mg/kg AMPH actually increases the synthesis of DA in the striatum[19,21]. The low doses used in this study (0.5 or 5 mg/kg AMPH) may be below the threshold required to produce neuronal depletion. This is an important finding, clinically, as AMPH is prescribed within this approximate dose range for chronic use in humans[41].

Administration of AMPH (5 mg/kg) to pregnant rats produced some intriguing results (Tables V and VI). At 3 weeks of age, male offspring of these dams exhibited normal striatal monoamine levels while the corresponding synthetic enzymes were significantly depressed. Results from similar studies have been variable[5,13,26,32], possibly due to wide discrepancies in doses and dosing regimens employed. In addition, many of the biochemical data reported to date for <u>in-utero</u> AMPH exposure have been data obtained from whole brain homogenates[5,26] which could easily mask the localized neurochemical changes that are detected in the present study.

Another important facet of these data is that they allow direct comparisons of effects on the offspring with effects on the treated dams. At a time that only enzymatic changes were present in the offspring (3 weeks), enzyme activities were normal in the dams but 5-HT, 5-HIAA, DA, DOPAC, and HVA levels were depressed. Since the data reflect only two time points after dosing, a complete explanation of the maternal-fetal interaction in response to chronic AMPH challenge during gestation is not possible. Enzyme activities in the dams may have been decreased during dosing and/or shortly thereafter but recovered by 3 weeks after dosing. A lag period in restoration of neurotransmitter levels after TH or TPH inhibition and recovery has

been reported in central dopaminergic[2] and serotonergic[30] systems, respectively. This could explain the depression in monoamines and metabolites observed here at 3 weeks. The offspring were exposed to AMPH in utero during the critical periods for development of the cerebral cortex (days 13-17 of gestation) and striatum (days 15-18 of gestation)[3]. Clearly, however, the neurochemical changes seen in these areas postnatally were minimal and temporary. The fetus may have a decreased sensitivity to this drug because of the immaturity of, or an increased plasticity in, the neuronal systems on which AMPH is known to act. Offspring and dams fully recovered from the drug insult, as all parameters were normal in both groups by 7 weeks post dosing. With the low doses of AMPH used in this chronic dosing regimen, the drug does not exhibit potent biochemical teratogenicity.

A comparison of the juvenile- and adult-treated males in the present study (5 mg/kg) shows that the neurotoxic effects in the hypothalamus appeared to be dependent upon the age of the animal at the time of exposure. At 16 hours post dosing, DA, 5-HT, and 5-HIAA were significantly depressed only in the adults. Preliminary data using 10 mg/kg per dose suggest that immature animals are not totally protected from the toxicity. Changes similar to those seen in the adults were produced in the juveniles at this higher dose. The dependence upon age for low-dose production of toxicity in the hypothalamus was not apparent in the other brain regions studied. Time- and dose-response studies are required to evaluate the importance of this finding. The present data underline, however, the importance of dose-response in the production of AMPH-induced neurotoxicity. Under the

conditions of this study, the severity of AMPH-induced toxicity did not increase dramatically in magnitude nor duration as a function of the age of the animal at the time of exposure.

Sex-related differences in behavioral sensitivity to AMPH have been studied in detail. Female rats show greater increases in activity levels and stereotypy than do males given equivalent doses[4,35]. AMPH is metabolized more rapidly in males, but when the administered dose is adjusted to give equivalent concentrations of AMPH in the brains of both sexes, females still show the greater behavioral response[4]. The present study extends these findings to a biochemical level. The females in this study (Table V) were more sensitive to chronic AMPH exposure than either of the treated male groups (juvenile or adult). The striatal depressions observed in the dams at 3 weeks post dosing (5 mg/kg) were, however, reversed by 7 weeks.

Intra-regional differences in sensitivity to AMPH were apparent, the hypothalamus showing a relative resistance, as reported elsewhere[1,33]. One possible explanation for the small magnitude and short duration of neurochemical changes in this area involves the availability of hydroxylase cofactor. Levine et al.[22] showed a strong correlation between total hydroxylase activity (TH + TPH) and the concentration of the natural cofactor, tetrahydrobiopterin (BH_4), in ten discrete regions of the rat brain. Most of the areas studied, including the striatum and cerebral cortex, exhibit hydroxylase activity linearly proportional to the amount of cofactor present. In the hypothalamus, however, there is a great excess of pterin for the amount of hydroxylase activity present. Our laboratory is currently

investigating the possibility that the neurotoxicity of the amphetamines involves depletion or inactiviation of this cofactor. If this theory is correct, areas such as the hypothalamus, containing a relative abundance of BH_4 would not be expected to show extensive depressions in hydroxylase activity (nor the corresponding depletions in neurotransmitters) in response to amphetamine challenge.

The two brain areas selected for their reported sensitivity to AMPH, the cortex and striatum, also showed very few significant changes. The changes that did occur were minor and short-lived. This cannot be attributed solely to a dose-response effect, as the highest dose used (10 mg/kg) has been shown to cause severe depletions when administered subacutely[38]. The present data support the idea that AMPH neurotoxicity can be greatly affected not only by dose level but also by duration of dosing.

The importance of the relative contributions of dose and duration of exposure on the neurotoxicity of amphetamines was illustrated in an early report by Koda and Gibb[17]. At least 10 or 15 mg/kg per dose of METH was necessary to decrease striatal TH when the drug was given every 6 hours for 8 doses (sacrifice 6 hours after the last dose). A similar paradigm has been used by many researchers to induce severe neurochemical depletions[1,10,14,33]. However, with continued dosing of METH (every 6 hours for 66 hours), TH activity in the striatum returned to normal levels by 60 hours, and appeared augmented by 72 hours, after the first dose[17]. These results were confirmed and extended by Kogan et al.[18] Data were also obtained from the substantia nigra, a site of dopaminergic cell bodies which project axons to

the striatum. With continued dosing of METH, TH activity was depressed earlier, and recovered faster, in the nigra than in the striatum[18]. Additional research is needed to elucidate the mechanism of dopaminergic system recovery with continued amphetamine exposure. This study lends preliminary support to the possibility that a similar recovery phenomenon occurs in the serotonergic system. Adult-treated males showed an apparent rebound increase in striatal TPH activity 3 weeks after the moderate dose (5 mg/kg, Table VI) and at 16 hours and 3 weeks after the highest dose (10 mg/kg, Table X).

Some of the effects observed in this study could have been caused by drug-induced changes in receptor populations. Several differences in receptor-mediated events occur as functions of dose level and duration of dosing of AMPH. High-dose subacute exposure decreases the number of dopaminergic agonist and antagonist binding sites[36] and the density of ^{3}(H)-DA uptake sites[39]. Conversely, lower dose, chronic AMPH exposure increases striatal DA binding[34]. Certainly, the neuronal system can respond differently at the molecular level to distinct types of drug challenge. Receptor changes must be included as offering plausible, if partial, explanations for the neurochemical effects reported here.

A single dose of 9.2-36.8 mg/kg AMPH administered with iprindole, which greatly slows elimination of the former drug, causes persistent depletions in the striatal dopaminergic system[11,12,38]. This has been used as a model for the neurotoxic effects of continuous central AMPH exposure. Implantation of AMPH-releasing pellets for 7 days depletes striatal TH activity for as long as 110 days. If the

same absolute dose is administered in divided daily injections over 7 days, no change of enzyme activity occurs[9]. These reports and the present study lend support to the concept that neuronal damage caused by AMPH is at least partially dependent upon an extended persistence of the drug in the brain. The greatest reported neurotoxicity caused by the amphetamines occurs after frequent administration or in the presence of a metabolic inhibitor that increases the brain half-life of the parent compound. Toxicity is greatly attenuated, however, in the chronic dosing regimen used here.

The central serotonergic and dopaminergic systems examined in this study appear to compensate for chronic, intermittent AMPH challenge, thereby reversing many of the neurotoxic effects that occur after short-term exposure.

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EFFECTS OF CHRONIC AMPHETAMINE ON CENTRAL SEROTONERGIC AND DOPAMINERGIC SYSTEMS IN RATS OF VARIOUS AGES

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